Scanning Electron Microscopy

Volume 1986 | Number 2 Article 36

5-30-1986

Changes in Absorptive Surfaces of Rat Visceral Yolk Sac with Increasing Gestational Age

W. P. Jollie
Medical College of Virginia

SCANNING ELECTRON MICROSCOPY/1986/II (Pages 661-669)
SEM Inc., AMF O'Hare (Chicago), IL 60666-0507 USA

0586-5581/86$1.00+05

CHANGES IN ABSORPTIVE SURFACES OF RAT VISCERAL YOLK SAC WITH INCREASING GESTATIONAL AGE

W. P. Jollie*

Department of Anatomy
Medical College of Virginia/Virginia Commonwealth University
Richmond, Virginia 23298

(Received for publication February 18, 1986: revised paper received May 30, 1986)

Abstract

The free surface of the endodermal epithelial lining of the rat visceral yolk sac was examined by scanning electron microscopy at three stages of pregnancy, viz., 12, 17 and 22 days (birth typically occurring on the 23rd day). Additionally, by ultrasonic vibration of tissues that had been subjected to prolonged osmium fixation, the epithelium was removed and such microdissected membranes similarly were examined.

With increasing gestational age the free surface of the epithelium underwent a relative increase in absorptive area by three mechanisms: formation of increasingly complex villous projections of the visceral yolk sac as a whole, a doming of the individual epithelial cells, and an increase in length and number of microvilli for each such cell. The resultant increase in surface area, however, could not be correlated with the onset of receptor mediated endocytosis at the latest stage.

The basement membrane underlying the lining epithelium of the yolk sac easily was revealed by ultrasonication of well-fixed tissues; it was most fragile at 12 days at which time the procedure apparently either removed the lamina lucida of the basal lamina, exposing a fibrillar component of the lamina densa, or removed the entire basal laminar component of the basement membrane, exposing an underlying reticular lamina. At 17 days the basement membrane at the cores of denuded villi showed the negative impressions of the epithelial cells that had been removed; and at 22 days, when the membrane is known to be permeable to protein transfer, it was smooth, featureless, and appeared most durable.

KEY WORDS: Visceral Yolk Sac, Rat Placenta, Absorptive Surfaces, Microdissection by Ultrasonication, Increasing Gestational Age.

*Address for Correspondence:
Department of Anatomy, Medical College of Virginia
Box 709, MCV Station
Richmond, VA 23298-0001
Phone No. (804)786-9624

Introduction

More than half a century ago it was realized that the inner, or visceral, wall of the yolk sac of rodents (a fetal membrane which in these forms enlarges completely to fill the extraembryonic celomic cavity between the amnion and chorion) serves as an alternate route for maternofetal exchange (Everett, 1933; 1935). Over the years the visceral yolk sac has been shown to absorb a variety of materials, principally proteins, from either the lumen of the yolk sac or the uterine cavity (with which, toward the end of pregnancy, the yolk-sac lumen becomes confluent) (Luse, 1958; Anderson and Leissring, 1961; Leissring and Anderson, 1961; Deren et al., 1966; Jollie and Jollie, 1966; Lambson, 1966; Padykula, et al., 1966; Beck et al., 1967; Carpenter and Ferm, 1969; Jollie and Seibel, 1970; King and Enders, 1970; Slade, 1970; Wild, 1970; Wild, 1973; King, 1974; and Seibel, 1974). More recently the visceral yolk-sac placenta of the rat has been shown as well to be the exclusive site for prenatal transport of serum proteins from mother to young, in particular the maternal antibodies that impart immunity to the newborn until the young can develop its own immunological competence (Kraehenbuhl et al., 1979; LaLiberté et al., 1981; Jollie, 1981; LaLiberté et al., 1984; and Jollie, 1985). The antibody initially is absorbed by receptor mediated endocytosis from the yolk-sac cavity/uterine lumen into the apical surfaces of the endodermal epithelial cells that line the confluent space (ibid.). In the rat, however, absorption of proteins into this membrane, including autologous antibodies, also can be affected by fluid phase endocytosis. This separate mechanism leads not to transport, but to degradation of the protein (Mucchielli et al., 1983; Jollie, 1985 and 1986). The rates of the two endocytotic mechanisms have been shown to vary independently from one another with increasing gestational age (Jollie and Triche, 1971; Jollie, 1981 and 1985). Indeed, receptor mediated endocytosis of antibody (which results in transport) occurs at the end of pregnancy, i.e., at the only time when physiologically placental transport of immunity has been shown to occur (Mayersbach, 1958).

The purpose of the present investigation has been to examine the outer surface of the rat visceral yolk-sac placenta by scanning electron microscopy at increasing gestational ages and to correlate changes in the fine structure of this absorptive surface with known changes in membrane function. Additionally, since materials absorbed by receptor mediated endocytosis must be transcytosed through the epithelial cells and absorbed across the underlying basement membrane before traversing the endothelium of vitelline capillaries to reach the fetal circulation, this basement membrane - also an absorptive surface - similarly was examined by scanning electron microscopy. To this end, visceral yolk-sac tissues were microdissected by a modification of the ultrasonic vibration method of Highison and Low (1982) for SEM examination of denuded basement membrane.

Materials and Methods

Sprague-Dawley rats (Charles River Breeding Laboratories) were selected at 12, 17, and 22 days of pregnancy, three specimens for each gestational age. Since this species exhibits a 23-day gestation period, these particular stages represent midpregnancy, half way through the second half of pregnancy, and the day before delivery. With the animals under deep nembutal anesthesia, the abdominal cavities were opened, the uteri were exposed, gestation sacs were opened, and visceral yolk-sac membranes were dissected free and transferred directly to fixative.

For SEM of the free surface of the endodermal epithelial membrane, tissues were fixed in 1.0% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M phosphate buffer with 0.01% $CaCl_2$, adjusted to pH 7.4 at room temperature for two hours, washed in the same buffer with 5% sucrose, postfixed in 2% OsO_4 in 0.1 M phosphate buffer and 0.01% $CaCl_2$ at pH 7.4 and 4°C, dehydrated with ethanol in ascending series, stored in butanol, and critical-point-dried with liquid CO_2 in a Polaron critical point drying apparatus, model E3000. For SEM of the basement membrane which underlies this epithelium, the yolk-sac membranes were fixed directly in the cold osmium textroxide solution for five days (a procedure that renders the tissue brittle and, accordingly, more susceptible to selective microdissection by ultrasonic vibration). Subsequently they were dehydrated in an ascending series of cold acetone and stored for five days or more in absolute acetone at 4°C. They were microdissected in ice-cooled absolute acetone by sonication at 55kHz for either 10 or 20 minutes in a Model B-221 Bransonic Ultrasonic Unit. Twenty minute sonication resulted in "cleaner" basement membranes i.e., with little or no residual epithelium adherent to them. On the other hand, twelve-day tissues that were sonicated for twenty minutes dissociated completely. Microdissected membranes were critical-point-dried in liquid carbon dioxide in a Ladd critical point dryer.

All tissues were mounted on studs, gold-coated in an Eiko IB-2 Ion Coater and examined in an Hitachi Hiscan 500 at 20 kV.

Results

Even at the light microscopic level - indeed, almost with the naked eye - the intact visceral yolk-sac placenta is characterized by bearing irregular villous projections around the root of the umbilical cord where the yolk sac comes into anatomical relationship with the fetal surface of the chorioallantoic placenta. It is this villous portion of the visceral membrane that functions as a placenta. [In fact, the maternal blood sinuses at the fetal surface of the chorioallantoic placenta constitute the maternal blood compartment of the yolk-sac placenta; the fetal compartment of the latter membrane, as mentioned above, is within a peripheral vitelline circulation which courses through these villi.] In an en face view of the membrane by SEM, the villi of the visceral yolk sac, however, appear more like undulating, often anastomosing ridges of considerable thickness (they often are considerably wider than the recesses between them); such ridge-like villi are well developed at 17 days (Figure 1) and at low magnification appear no more elaborately developed at 22 days (Figure 2); however, they are poorly developed in the 12-day visceral yolk sac where they appear unlike villi at all (i.e., they are not like tufts of hair) but as low ridges (lower than they are wide: Figure 3). The capillary network of the peripheral vitelline circulation, readily apparent in sectioned material, is not visible by SEM of the free surface of the membrane. The thickness of the epithelium effectively masks underlying blood vessels.

At all stages that were examined, individual endodermal epithelial cells on the villi themselves appear highly domed, each cell projecting a convexity of cytoplasm into the yolk-sac cavity (or, in 22-day tissue into the cavity of the uterus). These cells are most highly domed at 22 days at which stage they appear separated apically by deep gutters (Figures 4&5); they are less prominently domed at 17 days (Figure 1) and least domed at 12 days (Figure 3). In the latter instance, the cells at the periphery of the villous region, where the ridge-like villi also are least marked, appear quite flat and intercellular boundaries are indistinct (Figures 6&7). At all three stages that were examined the apex of each endodermal epithelial cell was seen to bear numerous microvilli, each about 0.75 μm long, which at fairly low magnification with SEM impart to the tissue a velvety appearance (Figures 4-7). They appear most densely packed at 22 days. At the two earlier stages the microvilli appear of uniform length; in the 22-day yolk sac occasionally giant microvilli (three-four times longer) were interspersed (Figure 5).

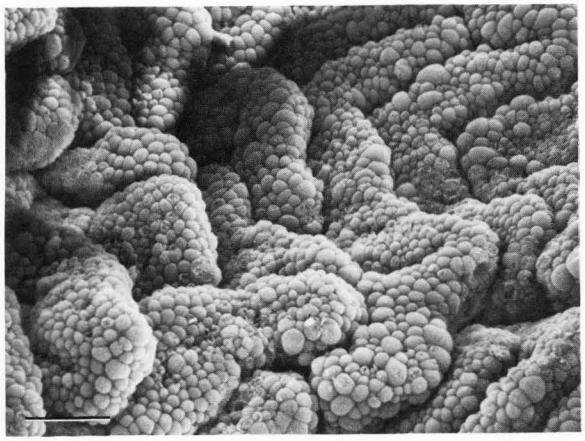

Fig. 1. 17-day visceral yolk-sac endodermal epithelial surface. Ridge-like villi and domed apical cell surfaces are apparent at low magnification. Bar = 50 μm.

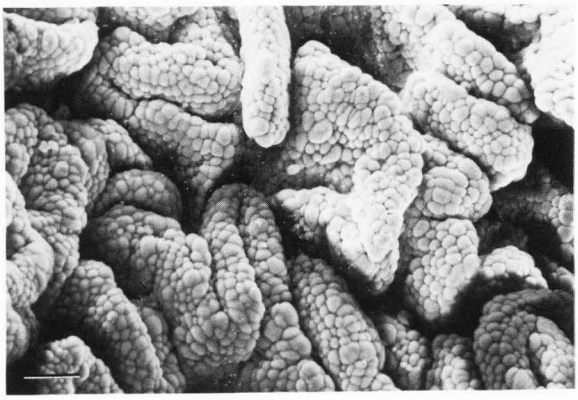

Fig. 2. 22-day epithelial cell surface. The tissue looks very much like 17-day visceral yolk sac. Bar = 50 μm.

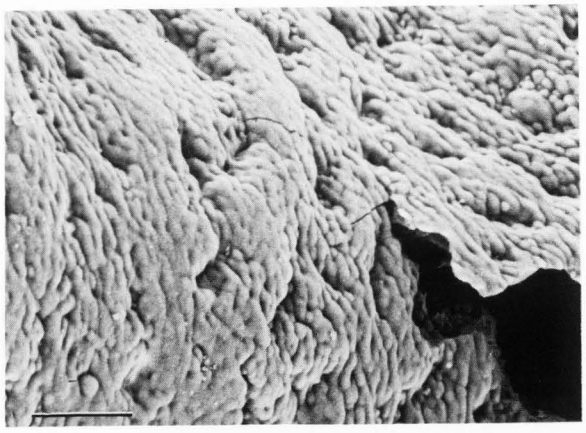

Fig. 3. 12-day yolk-sac membrane in surface view. Villi are poorly developed; and, since they are not highly domed, cells are not clearly outlined. The cut at lower right is where the membrane was separated from its attachment to the chorioallantoic placenta. Bar = 50 μm.

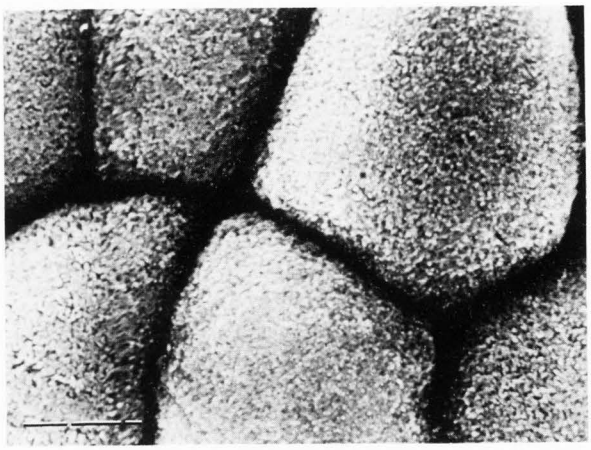

Fig. 4. The apical surfaces of several highly domed endodermal epithelial cells at 22 days. Black dots between the densely packed microvilli are thought to represent the recessed pits of endocytotic invaginations. Bar = 5 μm.

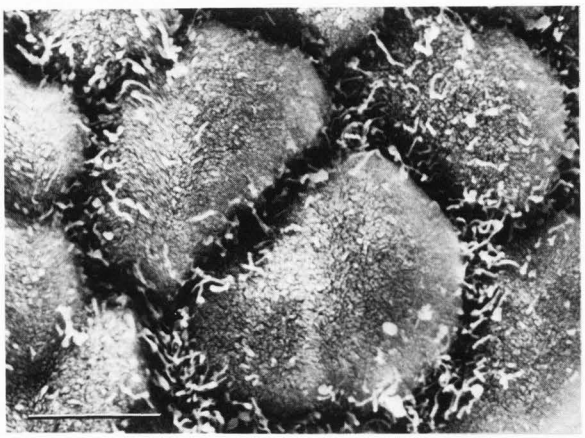

Fig. 5. Domed epithelial cells at 22 days. Note the irregular large microvilli among shorter, densely packed ones. Bar = 5 μm.

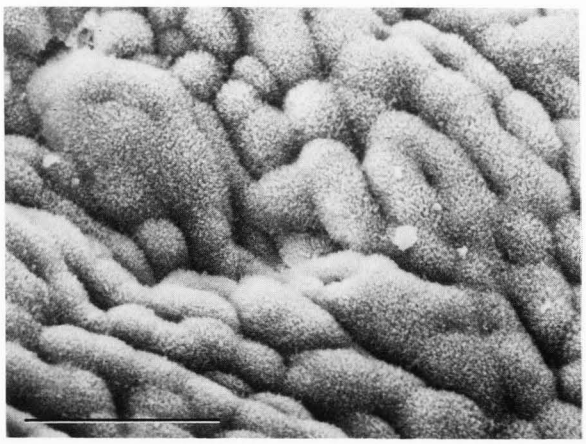

Fig. 6. At 12 days microvilli are short and few in number. Bar = 25 μm.

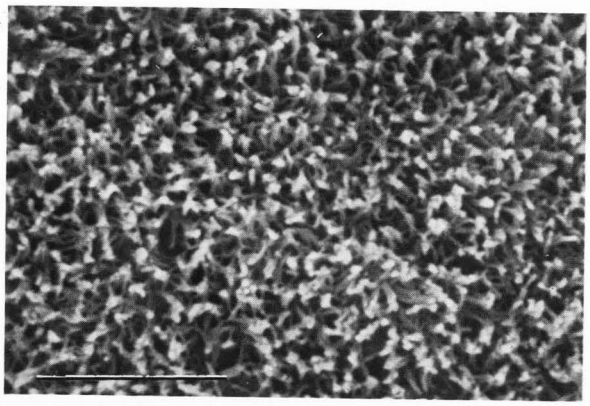

Fig. 7. At higher magnification, microvilli on 12-day yolk-sac epithelium appear few. Note apparent holes (active endocytosis). Bar = 5 μm.

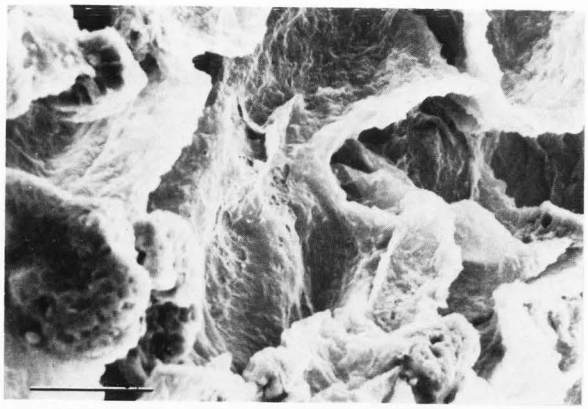

Fig. 8. On a 17-day ultrasonicated visceral yolk sac, the exposed basement membrane shows the negative images of the basal aspects of denuded epithelial cells. Clusters of cells are still attached at left. Bar = 50 μm.

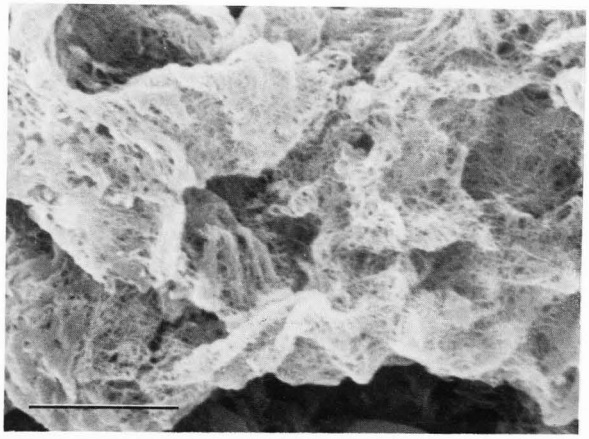

Fig. 9. The denuded basement membrane of the 12-day visceral yolk sac appears to be pierced by many small holes. Bar = 5 μm.

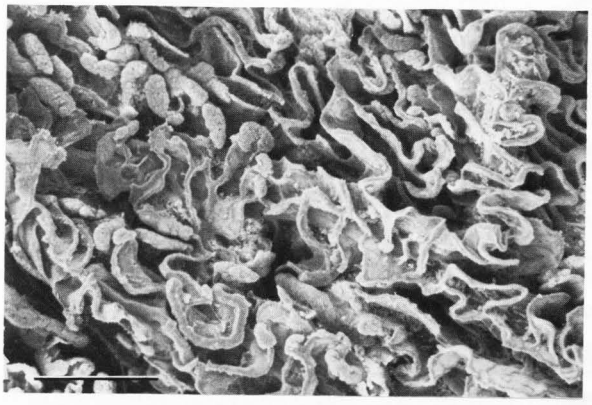

Fig. 10. At low magnification the denuded basement membrane of the 22-day yolk sac reveals the complexity of villous cores. Bar = 500 μm.

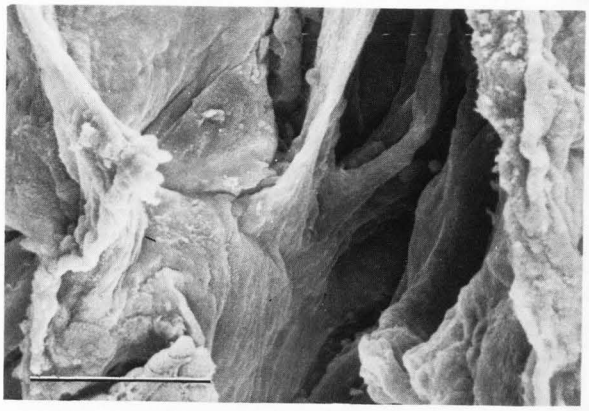

Fig. 11. At higher magnification the 22-day denuded basement membrane over the cores of ridge-like villi appears relatively smooth. A cluster of adherent cells appears at lower left. Bar = 50 μm.

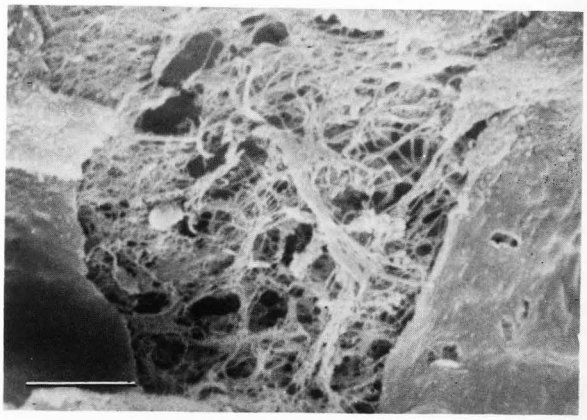

Fig. 12. In ultrasonication tissues, a meshwork similar to that identified in 12-day visceral yolk sacs occasionally appears through torn holes in the smooth denuded basement membrane at later stages (in this case, at 22 days of gestational age). Bar = 5 μm.

In visceral yolk sacs that have been microdissected by ultrasonic vibration, the membrane is less rigid (turgid) and after critical point drying it tends to collapse into wrinkles (Figure 8). It often is difficult to differentiate between such wrinkles and the cores of individual villi. A differentiation between such deformation which occurs in preparation of the tissue and naturally occurring villous ridges is particularly difficult in the 12-day membrane (Figure 9). The presence of undulating, anastomosing ridge-like villous cores is most apparent in the 22-day sonicated membrane, where the denuded basement membrane is highly and elaborately folded into high ridges that give the surface a somewhat honeycomb appearance (Figure 10). The outer surface of the denuded basement membrane itself, however, is relatively smooth and "clean" at this latest stage (Figure 11). At 17 days, though imperforate, often it is deformed by being marked up with regular, shallow concavities which presumably fit around the basal aspects of the denuded epithelial cells (Figure 8). At the earliest stage, viz., 12 days of pregnancy, the outer surface of the microdissected visceral yolk-sac membrane appears undeformed by the cells that formerly overlay it; however, it appears highly perforated with countless holes of about 250 nm in diameter. At high magnification (Figure 9) it appears to be a sheet made up of a meshwork of interlacing fibrils. Within this lattice matrix perforations, as gaps in the meshwork, regularly are spaced. In tissues autopsied at later gestational ages and subsequently ultrasonicated for 20 minutes, occasionally ruptures in the membrane reveal a substructure similar to the denuded membrane at 12 days (Figure 12).

Discussion and Conclusions

Throughout pregnancy the absorptive surface of the visceral yolk-sac membrane through which receptor mediated endocytosis occurs during placental transport continues to increase in area. The entire gestation sac enlarges as pregnancy advances, of course, but relative to this continued overall growth, the absorptive surface of the placental region of the yolk sac increases more. Three mechanisms serve to increase the region of absorptive surface of the endodermal epithelium relative to overall placental area: the formation of villi, the doming of the apices of the individual epithelial cells, and the presence of microvilli at these apical surfaces. The villi [which first appear at 12 days; the 11-day yolk sac lacks them] are lower than they are wide with shallow depressions between them at 12 days; they are as well developed by 17 days as they are at 22, i.e., as developed as they ever will be. Doming of the free surface of the free surface of the endodermal cells, however, increases markedly as pregnancy advances. At 12 days, in surface view the separation of cells sometimes is difficult to discern; it is readily apparent at 17 days at which time individual cell apices are distinct. At 22 days deep gutters separate these cells. Whether there is an increase in the number of microvilli per epithelial cell cannot be determined with certainty without precise morphometric analyses of scanning micrographs. However, even a cursory examination of high magnification scanning views of cell apices reveals that microvilli are longer, but fewer at 12 days, and more numerous at 17 days, shorter and so abundant that they appear densely packed at 22 days (compare Figures 4 and 5 with Figures 6 and 7). These observations correlate well with data from transmission electron microscopic studies of visceral yolk-sac epithelium where at 12 days microvilli are about 1.0 μm long and as little as half that length at 22 days (pictured, though not cited by Jollie, 1985).

Although it is tempting to suggest that an increase in the absorptive surface of the epithelium of the placental membrane at the three stages that have been observed correlates directly with an increase in absorptive capacity, no evidence has been presented here or elsewhere to suggest that either the height or number of villi nor, indeed, the degree of doming of cellular apical surface is in any way related to the rate of activity of receptor mediated endocytosis (Jollie and Triche, 1971; Jollie, 1985). By transmission electron microscopy in these earlier studies, receptor sites have been identified at the fundi of some of the recesses between microvilli, not on them. It is at such sites that, as a result of clathrin assembly, initial deformation of the membrane occurs by the localized invagination that ultimately leads to a pinching off of a closed microvesicle (Pearse, 1976). In all likelihood the "holes" in the free surfaces of the epithelial cells at all stages represent such invaginating pits (Figures 4 and 7). Unfortunately, however, it has been shown that uptake of proteins also occurs by fluid phase (i.e., non-absorptive) endocytosis at the same surface and into a system of subapical canaliculi whence it is transferred to secondary lysosomes for degradation (LaLiberté et al., 1981; Mucchielli et al., 1983; Jollie, 1985). Fluid phase and receptor mediated endocytosis vary independently of one another; the latter process is active at 22 days, the former earlier on. Unfortunately the initial uptake of material in both cellular mechanisms is by deformation of the apical plasma membrane into recessed pits; the initial steps of both processes probably would look identical by scanning electron microscopy. Accordingly, from the results of this study one cannot tell whether a "hole" represents initiation of receptor mediated or fluid phase endocytosis.

Since only in the case of receptor mediated endocytosis is the material that has been taken up transferred ultimately to the vitelline (fetal) circulation, this process results in

passage of the protein through the basement membrane that separates the endodermal epithelium from the connective tissue core of the villus [which in turn, contains the vitelline capillaries]. The external (epithelial) surface of this basement membrane readily is exposed by microdissecting the tissue with ultrasonic vibration by a modification of the method of Highison and Low (1982) and Low and McClugage (1984). Ultrasonicated basement membranes present three different appearances at the three gestational ages that were examined: smooth and largely featureless at 22 days, scalloped into depressions as a kind of mold for the bases for the endodermal epithelial cells at 17 days, and composed of a meshwork of fine fibrils at 12 days. Like basement membranes generally, that of the endodermal epithelium [the visceral basement membrane of Wislocki and Padykula (1953)] with transmission electron microscopy seems to be composed of a basal lamina apposed to the epithelium and a reticular lamina against the connective tissue compartment beneath. Again, like other basement membranes, the basal lamina, in turn, is seen to consist of a lamina densa (against the reticular lamina) and a lamina lucida, an optically empty zone separating the dense lamina from the basal plasmalemma of the cells of the epithelial sheet (Lambson, 1966; Jollie, 1984). Which layer of the three, reticular lamina, lamina densa (of the basal lamina), or the lamina lucida, were exposed by ultrasonication in the present study is not certain, nor, indeed, is it altogether clear if the same layer was exposed at three different gestational ages. The 17-day and 22-day exposed "basement membranes" were enough alike to suggest that they were, indeed, the same layer. The fibrillar nature of the membrane in the 12-day yolk-sac placenta, however, suggests in this case either that the lamina lucida of the basal lamina had been removed, revealing a lamina densa with a fibrillar component, or that the entire basal lamina had been removed and a reticular lamina was exposed to scanning microscopy. Since at this early stage a reticular lamina has not been identified by transmission electron microscopy (unpublished observations of the author), the former interpretation seems more likely. Furthermore, even basement membranes at the 17-day stage occasionally were seen to have small areas of rupture through which a similar network of interlocking fibrils was visible immediately beneath a single layer. At 22 days the basal lamina has been shown by transmission electron microscopy to have replicated so that several - as many as six - such laminae densae separate the reticular lamina from the epithelium (Jollie, 1984).

Correlating changes in the structure of the basement membrane - from newly formed and delicate to fully formed and to replicated - with known changes in its permeability to transport is difficult to assess from the data that have been presented here. One would expect the membrane to decrease in permeability as it becomes less fragile, thicker and finally replicated. Yet it is the replicated membrane that has been shown to be permeable to immunoglobulins (Jollie, 1985). Presumably these proteins - like the exogenous tracer proteins, ferritin and horseradish peroxidase the passage of which has been observed directly (Lambson, 1966; Seibel, 1974) - dissolve into the membrane and pass through it by diffusion. It would seem, then, that the permeability of the visceral yolk-sac membrane as a whole to the placental transport of proteins is not determined by the permeability of the basement membrane layer. Instead, the limiting layer to transport appears to be the epithelium.

References

1. Anderson JW, Leissring JC. (1961). The transfer of serum proteins from mother to young in the guinea pig. II. Histochemistry of tissues involved in prenatal transfer. Am. J. Anat. 109, 157-174.
2. Beck FJ, Lloyd JB, Griffiths I. (1967). A histochemical and biochemical study of some aspects of placental function in the rat using maternal injection of horseradish peroxidase. J. Anat. 101, 461-478.
3. Carpenter SJ, Ferm VH. (1969). Uptake and storage of Thorotrast by the rodent yolk-sac placenta: an electron microscopic study. Am. J. Anat. 125, 429-456.
4. Deren JJ, Padykula HA, Wilson TH. (1966). Development of structure and function in the mammalian yolk sac. II. Vitamin B_{12} uptake in rabbit yolk sacs. Devel. Biol. 13, 349-369.
5. Everett JW. (1933). Structure and function of the yolk-sac placenta in Mus norvegicus albinus. Proc. Soc. Exp. Biol. Med. 31, 77-99.
6. Everett JW. (1935). Morphological and physiological studies of the placenta of the albino rat. J. Exp. Zool. 70, 243-285.
7. Highison GJ, Low FN. (1982). Microdissection by ultrasonication after prolonged OsO_4 fixation: a technique for scanning electron microscopy. J. Submicr. Cytol. 14, 161-170.
8. Jollie WP. (1981). Visualization of antibody transport in rat visceral yolk-sac placenta. In "Proceedings of the Electron Microscope Society of America", G.W. Bailey (ed.), 246-247, Claitor's: Baton Rouge, LA.
9. Jollie WP. (1984). Changes in the fine structure of rat visceral yolk-sac placenta during prolonged pregnancy. Am. J. Anat. 171, 1-14.
10. Jollie WP. (1985). Immunocytochemical localization of antibody during placental transmission of immunity in rats. J. Reprod. Immunol. 7, 261-274.
11. Jollie WP. (1986). Ultrastructural studies of protein transfer across rodent yolk sac. Placenta 7, 263-281.

12. Jollie WP, Jollie LG. (1966). Visualization of transport of electron opaque colloids in placental membranes of the rat. In "Proceedings of the Electron Microscope Society of America," C.J. Arceneaux (ed.), 423-424, Baton Rouge, LA: Claitor's Publ. Div.
13. Jollie WP, Seibel W. (1970). Fine structural observations on transport mechanisms in visceral yolk-sac placenta of the rat. In "Proceedings of the Electron Microscope Society of America,", C.J. Arceneaux (ed.), 50-51, Baton Rouge, LA: Claitor's Publ. Div.
14. Jollie WP, Triche TJ. (1971). Ruthenium labeling of micropinocytotic activity in the rat visceral yolk-sac placenta. J. Ultrastruct. Res. 35, 541-553.
15. King BF. (1974). An electron microscopic investigation of the surface coat of visceral yolk-sac endoderm cells in the guinea pig. Anat. Rec. 180, 299-308.
16. King BF, Enders AC. (1970). Protein absorption and transport by the guinea pig visceral yolk-sac placenta. Am. J. Anat. 129, 261-288.
17. Kraehenbuhl JP, Bron C, Sordat B. (1979). Transfer of humoral secretory and cellular immunity from mother to offspring. Curr. Topics Pathol. 66, 105-157.
18. LaLiberté F, Mucchielli A , Ayraud N, Masseyeff R. (1981). Antibody transfer mechanisms from mother to fetus across rat yolk-sac endoderm. Am. J. Reprod. Immunol. 1, 345-351.
19. LaLiberté F, Mucchielli A, LaLiberté MF. (1984). Dynamics of antibody transfer from mother to fetus through the yolk-sac cells in the rat. Biol. Cell. 50, 255-262.
20. Lambson RO. (1966). An electron microscopic visualization of transport across rat visceral yolk sac. Am. J. Anat. 118, 21-32.
21. Leissring JC, Anderson JW. (1961). The transfer of serum proteins from mother to young in the guinea pig. I. Prenatal rates and routes. Am. J. Anat. 109, 149-155.
22. Low FN, McClugage SG. (1984). Microdissection by ultrasonication: scanning electron microscopy of the epithelial basal lamina of the alimentary canal in the rat. Am. J. Anat. 169, 137-147.
23. Luse SA. (1958). The morphologic manifestations of uptake of materials by the yolk sac of the pregnant rabbit. In "Gestation: Transactions of the Fourth Conference." C.A. Villee (ed.), 115-141. New York: Josiah Macy, Jr. Foundation.
24. Mayersbach HV. (1958). Zur Frage des Proteinüberganges von der Mutter zum Fötus. I. Erfunde an Ratten am Ende der Schwangerschaft. Z. Zellforsch. 48, 479-504.
25. Mucchielli A, LaLiberté F, LaLiberté MG. (1983). A new experimental method for the dynamic study of the antibody transfer mechanisms from mother to fetus in the rat. Placenta. 4, 175-183.
26. Padykula HA, Deren JJ, Wilson TH. (1966). Development of structure in the mammalian yolk sac. I. Developmental morphology and vitamin B_{12} uptake in the rat yolk sac. Devel. Biol. 13, 311-348.
27. Pearse BM. (1976). Clathrin: a unique protein associated with intracellular transfer of membrane by coated vesicles. Proc. Nat. Acad. Sci. U.S.A. 73, 1255-1259.
28. Seibel W. (1974). An ultrastructural comparison of the uptake and transport of horseradish peroxidase by the rat visceral yolk-sac placenta during mid and late gestation. Am. J. Anat. 140, 213-236.
29. Slade BS. (1970). An attempt to visualize protein transmission across the rat visceral yolk sac. J. Anat. 107, 531-545.
30. Wild AE. (1970). Protein transmission across rabbit foetal membranes. J. Embryol. Exp. Morphol. 24, 313-330.
31. Wild AE. (1973). Transport of immunoglobulin and other proteins from mother to young. In "Lysosomes in Biology and Pathology" Part 3. J.T. Dingle (ed.), 179-215. Amsterdam: North Holland.
32. Wislocki GW, Padykula HA. (1953). Reichert's membrane and the yolk sac of the rat investigated by histochemical means. Am. J. Anat. 92, 117-151.

Discussion with Reviewers

F.N. Low: In our initial experiences with prolonged exposure to osmic acid we got the impression that no measurable increase in brittleness occurred after 48 hours exposure. What is the rationale of the five-day immersion used in your experiments?

Author: I do not believe that prolonged immersion from 48 hours to five days necessarily increased brittleness of the tissue; it was simply convenient to keep them for a maximum of five days. As a matter of fact, 17-day tissues that had been exposed for only 3 days showed no differences in the results of ultrasonication.

F.N. Low: The shallow concavities presumably traceable to the basal surfaces of denuded epithelial cells (Figure 8) were noticed in our preparations of esophagus from which stratified epithelium had been removed (Am. J. Anat., 169: fig. 1, p. 139) but not elsewhere in the alimentary canal. Have you noticed comparable variability in your preparations and, if so, can you interpret this?

Author: I have not seen a comparable regional variability in the yolk sac, but then I have examined only the villous portion of the visceral layer of this membrane. There was, of course, variability in the configuration of the basement membrane, as reported here, with increasing gestational age. The concavities were seen only in 17-day denuded tissues.

S.G. McClugage: As I look carefully at Figure 9, I see flattened opaque fragments that appear to be basement membrane remnants. Since the 12-day samples appear to be more vulnerable to sonication damage, did you sonicate any of these samples for only a short period of time (less than 1 minute) in order to minimize such damage? Furthermore, did you try soaking your fresh samples in a 1% boric acid solution for one hour or less to loosen the epithelium to an extent that the basement membrane, if there is one, could be exposed without sonication? Your point is well-taken that the observations by SEM of the fibrillar nature of the basement membrane are difficult to interpret. Since the lamina densa is composed of Type IV collagen arranged in a network fashion (J. Cell Biol. 1983; 97: 1524-1537), it may be difficult to distinguish this from the reticular lamina by SEM if the lamina rara is absent or removed.

Author: Tissues were not ultrasonicated for less than 1 minute, nor pretreated for ultrasonication in boric acid solution. Fetal disintegration of 12-day tissues when ultrasonicated for 20 minutes was, of course, an unexpected finding. A sufficient number of entire yolk sacs at this earliest stage had been sampled, however, to allow trying a 10-minute ultrasonication procedure. Fortunately it produced the results that are reported. By TEM a subepithelial basement membrane is visible in the 12-day visceral yolk sac. It is delicate (i.e., thin), stains poorly and appears to lack a reticular lamina. To my knowledge the pattern of the Type IV collagen fibrils in the lamina densa of basement membranes has not been determined.

S.G. McClugage: You appear to consider the basement membrane and the epithelium of the visceral yolk-sac membrane as two independent morphologic entities, each with the potential for acting as a selective barrier for the passage of substances. In view of what is thought about the effect that the basement membrane or the extracellular matrix may have on cells particularly in embryonic tissues, can we then think of such a sharp dichotomy? Cannot some of the constituents of the basement membrane or extracellular matrix such as fibronectin or other glycoproteins be orchestrating some of the epithelial changes you describe in your paper? Cannot even receptor-mediated endocytosis be under the influence of matrix molecules?

Author: The point is well taken if one considers the possible effects of subepithelial basement membrane and the connective tissue extracellular matrix beneath it on exocytosis of materials through the basal plasmalemma. In the case of the parietal yolk sac, the unusually large subepithelial basement membrane, termed Reichert's membrane, appears to transport independently from the epithelium; moreover, it is semipermeable (Jollie, 1968, Am. J. Anat., 122: 513). In the visceral membrane, however, it appears to be epithelium and basement membrane together that constitute a "barrier" to free exchange of materials between the uterine lumen/yolk-sac cavity and the connective tissue compartment with its fetal capillary network. Whether in transporting epithelia exocytosis is receptor-mediated or not, to my knowledge, is unknown. It is difficult to imagine how extracellular matrix in the connective tissue compartment of the yolk sac could orchestrate receptor-mediated endocytosis through the apices of the epithelial cells, except perhaps by "reversed transcytosis" of basal plasmalemma to plasmalemma at the free surface of the cells. Such a reverse transport mechanism in this system has not been demonstrated.

Reviewer III: Between the 17th and 22nd days of gestation in the rat the antimesometrial decidua, trophoblast and parietal wall of the yolk sac normally deteriorate and rupture. As a result the yolk-sac cavity and uterine lumen become confluent. Might this event correlate with the peaking of surface area increasing specializations of the visceral yolk-sac endoderm cells during the same gestational period?

Author: The parietal yolk sac over the fetal surface of the chorioallantoic placenta does not rupture, but continues without profound morphologic or chemical change until term, serving as the only separation between maternal blood in the labyrinthine sinuses of the chorioallanotis and the yolk-sac cavity or uterine lumen (Jollie, 1968, Am. J. Anat., 122: 513). This persistent "placental parietal yolk sac" is readily permeable to protein (ibid). Since an intact "capsular parietal yolk sac"/antimesometrial decidua complex appears to present no physiologic barrier to protein transfer between maternal blood and yolk-sac cavity, or, after 19 days of pregnancy the confluent yolk-sac uterine cavity, by this route one would expect little if any correlation between rupture of the membranes and absorptive activity. Cited previous work (Jollie and Triche, 1971) has shown a peak in micropinocytotic activity (presumably fluid-phase endocytosis) at 12 days of pregnancy; more recent cited evidence (Jollie, 1986) suggests that receptor-mediated endocytosis of immunoglobulin is most abundant just before term.

Reviewer III: Would you please compare your findings on the surface changes of rat visceral yolk sac endoderm cells at increasing gestational ages with those presented in a similar SEM study of the mouse yolk-sac placenta by Pendergrass, Reams and Scott (J. Submicros. Cytol., 14: 279-284, 1982)?

Author: The 1982 paper by Pendergrass et al. was designed "to present an SEM description of the inner mesothelial surface of the visceral yolk sac, the endodermal surfaces of the visceral and parietal yolk sac and related fetal surfaces...in the mouse." The SEM of the absorptive endodermal surface of the villous (i.e., absorptive) portion of the visceral yolk sac was described in a single sentence, as follows: "At all days cells of the villous area are covered with abundant microvilli which are either bleb-shaped or elongated." No changes with increasing gestational age were reported; no attempt was made to correlate changes in fine structure with functional changes; descriptions of the membrane following microdissection by ultrasonication were not provided.

G.J. Highison and F.D. Tibbitts: Why were samples stored in butanol prior to critical point drying?

Author: Tissues were stored in butanol as they were collected, three yolk sacs from each of three animals at each of the three stages of pregnancy in order to collect a full series of tissues. Subsequently they were processed together.

G.J. Highison and F.D. Tibbitts: Are you aware of any differences in connective tissue composition of the 12-day sample which resulted in complete dissection versus 17 and 22-day samples?

Author: No. The chemical composition of the connective tissue core of the villi is unknown. In 12-day samples it is virtually absent, and peripheral capillaries of the vitelline circulation course directly beneath a delicate adepithelial basal lamina. With increasing gestational age a true connective tissue core develops and increases in amount.

G.J. Highison and F.D. Tibbitts: Is there any evidence of reticular fibers within the 12-day sample?

Author: In the villi themselves at 12 days no reticular fibers can be identified. A reticular lamina is not apparent by TEM.

G.J. Highison and F.D. Tibbitts: Have you noted structural (compositional) differences in other areas of the yolk sac?

Author: No. The only other area of rat yolk sac that has been examined by EM is the placental portion of the parietal yolk sac which has altogether a different composition. I have not examined non-villous visceral yolk sac.

A.K. Champlin: Is the villous nature of the yolk sac shown in these micrographs characteristic of the yolk sac in all places or just around the root of the umbilical cord?

Author: The villi are confined to an area around the root of the umbilical cord where the membrane comes into anatomical relationship with the fetal surface of the chorioallantoic placenta.

A.K. Champlin: Where is the evidence that the 11-day yolk sac lacks villi? It doesn't seem to be part of this presentation, nor is it referenced in the text.

Author: Classic descriptions of the yolk-sac placenta of the rat from the early work of Duval (1891. Journ. Anat. Physiol., 27:515) onwards have described the visceral membrane as bearing villi from 12 days of gestational age, at which time they are poorly developed, until term. I have made the same observation, but have not cited it as an unpublished observation in this paper.

A.K. Champlin: What are the relative rates of the two endocytotic mechanisms at the times at which the yolk sac was sampled?

Author: Fluid-phase endocytosis appears to be most active at 12 days, less so at 17 and least at 22 days. Conversely, receptor-mediated endocytosis of antibodies has been observed only at 22 days in our laboratory (cited in Jollie, 1986). Mucchielli et al. (1983), however, reported receptor-mediated endocytosis at 17 days in their experimental system (also rat). It may well be that rates vary according to concentration of ligand that is endocytosed. In particular for fluid-phase endocytosis there is evidence to suggest a direct correlation between concentration and endocytotic rates.